

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently Amended) A method of detecting an analyte, comprising:

(i) combining:

(a) an analyte;

(b) a first proximity member, comprising a first analyte-specific binding entity and a first oligonucleotide comprising a first portion wherein the first analyte-specific binding entity is capable of forming a complex with the analyte and is conjugated to the first oligonucleotide;

(c) a second proximity member, comprising a second analyte-specific binding entity and a second oligonucleotide comprising a portion that is capable of hybridizing to the first portion of the first oligonucleotide wherein the second analyte-specific binding entity is capable of forming a complex with the analyte and is conjugated to the second oligonucleotide comprising a portion that is capable of hybridizing to the first portion of the first oligonucleotide; and

(d) a hybridization blocker oligonucleotide at a concentration in excess of the concentration of the first and second proximity members, wherein the hybridization blocker oligonucleotide comprises a portion that is capable of forming a hybrid with the first portion of the first oligonucleotide to reduce hybridization between the first and second oligonucleotides and wherein the first and/or second analyte-specific binding entity is a protein;

(ii) forming a complex comprising the analyte, the first proximity member, and the second proximity member, and the hybridization blocker oligonucleotide that is hybridized with the first portion of the first oligonucleotide;

(iii) forming a hybrid by displacing the hybridization blocker oligonucleotide wherein the hybrid comprises the first portion of the first oligonucleotide and the portion of the second oligonucleotide, wherein the hybrid comprises a 3' terminus of the first or second oligonucleotide that may be extended;

(iv) extending the 3' terminus of the first or second oligonucleotide and producing an amplicon;

(v) amplifying the amplicon and producing an amplification product; and

(vi) detecting the amplification product, wherein detection of the amplification product allows-indicates detection of the analyte.

2. (Previously Presented) The method of claim 1, wherein the hybridization blocker oligonucleotide comprises a 3' sequence that is not complementary to the first oligonucleotide.

3. (Previously Presented) The method of claim 1, wherein the hybridization blocker oligonucleotide comprises a 5' sequence that is not complementary to the first oligonucleotide.

4. (Previously Presented) The method of claim 1, wherein the hybridization blocker oligonucleotide comprises a 3' sequence and a 5' sequence that are not complementary to the first oligonucleotide.

5. (Currently Amended) The method of claim 1, wherein the hybridization blocker oligonucleotide comprises a 3' cap that prevents 3' extension of the hybridization blocker oligonucleotide by a DNA polymerase.

6. (Currently Amended) The method of claim 1, wherein the hybridization blocker oligonucleotide forms a hybrid with the first portion of the first oligonucleotide wherein the hybridization blocker oligonucleotide contains bases that form a hybrid are complementary with all of the bases of the first portion of the first oligonucleotide comprising all of the bases of the first portion of the first oligonucleotide.

7. (Previously Presented) The method of claim 6, wherein the first portion of the first oligonucleotide is about 10 bases in length and the hybridization blocker oligonucleotide is about 18 bases in length.

8. (Currently Amended) The method of claim 6, wherein the length of the first portion of the first oligonucleotide is about the length of the [[entire]]first oligonucleotide.

9. (Withdrawn-Currently Amended) The method of claim 1, wherein the hybridization blocker oligonucleotide forms a hybrid with the first portion of the first oligonucleotide wherein the bases of the hybridization blocker oligonucleotide of the hybrid are comprising less than all of the bases of the first portion of the first oligonucleotide.

10. (Currently Amended) The method of claim 1, further ~~comprising adding~~
combining a deblocker oligonucleotide that is capable of reducing the hybridization between the
hybridization blocker oligonucleotide and the first oligonucleotide.

11. (Previously Presented) The method of claim 10, wherein the deblocker
oligonucleotide comprises a first portion that is capable of forming a hybrid with the portion of
the hybridization blocker oligonucleotide that is capable of forming a hybrid with the first
portion of the first oligonucleotide.

12. (Previously Presented) The method of claim 11, wherein the deblocker
oligonucleotide comprises a second portion that is capable of forming a hybrid with the portion
of the hybridization blocker oligonucleotide that does not form a hybrid with the first portion of
the first oligonucleotide.

13. (Currently Amended) The method of claim 1, wherein the hybridization blocker
oligonucleotide comprises a double-stranded portion, [[that]]wherein the double-stranded portion
is 3' of the portion of the hybridization blocker oligonucleotide that is capable of forming a
hybrid with the first portion of the first oligonucleotide.

14. (Original) The method of claim 13, wherein the double-stranded portion
comprises a hairpin loop.

15. (Cancelled)

16. (Withdrawn) The method of claim 10, wherein the hybridization blocker oligonucleotide hybridizes with the first portion of the first oligonucleotide after the addition of the deblocker oligonucleotide.

17. (Withdrawn-Currently Amended) The method of claim 10, wherein the hybridization blocker oligonucleotide ~~hybridizes~~ is added simultaneously with the deblocker oligonucleotide.

18. (Withdrawn) The method of claim 1, wherein the hybridization blocker oligonucleotide is added before the combination of the analyte and first and second proximity members.

19. (Withdrawn) The method of claim 1, wherein the hybridization blocker oligonucleotide is added after the combination of the analyte and first and second proximity members.

20. (Previously Presented) The method of claim 1, further comprising adding in step (i) a second hybridization blocker oligonucleotide at a concentration in excess of the concentration of the first and second proximity members, [[that]] wherein the second hybridization blocker oligonucleotide is capable of hybridizing to the portion of the second oligonucleotide wherein the portion of the second oligonucleotide is capable of forming a hybrid with the first portion of the first oligonucleotide, forming a complex in step (ii) further

comprising the second hybridization blocker oligonucleotide hybridized to the portion of the second oligonucleotide, and displacing in step (iv) the second hybridization blocker oligonucleotide.

21. (Previously Presented) The method of claim 1, wherein said amplifying the amplicon is by a method selected from the group consisting of polymerase chain reaction, strand displacement amplification, thermophilic strand displacement amplification, self-sustained sequence replication, nucleic acid sequence-based amplification, Q β replicase system based amplification, ligase chain reaction, and transcription mediated amplification.

22. (Currently Amended) The method of claim 1, wherein the hybridization blocker oligonucleotide reduces formation of the amplicon by hybridization of the first and second oligonucleotides prior to forming [[a]]said complex by a factor of at least 100-fold as detected in assay relative to amplicon formation without a hybridization blocker oligonucleotide.

23. (Currently Amended) The method of claim 22, wherein the hybridization blocker oligonucleotide reduces formation of the amplicon by a factor of at least 1000-fold as detected in assay relative to amplicon formation without a hybridization blocker oligonucleotide.

24. (Currently Amended) The method of claim 1, wherein the analyte is capable of being detected when the concentration of the analyte is at least about 1 pM.

25. (Previously Presented) The method of claim 1, wherein the analyte is capable of being detected when the concentration of the analyte is at least about 0.1 pM.

26. (Previously Presented) The method of claim 1, wherein the analyte is capable of being detected when the concentration of the analyte is at least about 0.01 pM.

27. (Previously Presented) The method of claim 1, wherein the detecting the amplification product is quantitative.

28. (Previously Presented) The method of claim 1, wherein the first or second analyte-specific binding entity is a protein complex.

29. (Previously Presented) The method of claim 28, wherein the protein complex comprises a first protein that is conjugated to the first or second oligonucleotide and a second protein that is capable of forming a complex with the analyte.

30. (Original) The method of claim 29, wherein the first protein is selected from the group consisting of Protein A and Protein G.

31. - 73. (Cancelled)

74. (Currently Amended) A method of detecting an analyte, comprising:
(i) combining:

(a) an analyte;

(b) a first proximity member, comprising a first analyte-specific binding entity and a first oligonucleotide comprising a first portion and a second portion comprising a 5' terminus wherein the first analyte-specific binding entity is capable of forming a complex with the analyte and is conjugated to the first oligonucleotide;

(c) a second proximity member, comprising a second analyte-specific binding entity and a second oligonucleotide comprising a first portion wherein the second analyte-specific binding entity is capable of forming a complex with the analyte and is conjugated to the second oligonucleotide, wherein the first portion of the first oligonucleotide is capable of hybridizing to the first portion of the second oligonucleotide and the first and/or second analyte-specific binding entity is a protein;

(ii) forming a complex comprising the analyte, the first proximity member, and the second proximity member, wherein the complex contains at least one hybrid comprising the first portion of the first oligonucleotide and the first portion of the second oligonucleotide, wherein the at least one hybrid comprises ~~[[a]]~~the 3' terminus of the second oligonucleotide that is capable of being extended to form a complement of the second portion of the first oligonucleotide;

(iii) extending the 3' terminus and producing an amplicon;

(iv) amplifying the amplicon and producing an amplification product; and

(v) detecting the amplification product, wherein detection of the amplification product ~~allows-indicates~~ detection of the analyte.

75. (Canceled)

76. (Currently Amended) The method of claim 74, further ~~comprising~~
~~adding~~combining in step (i) a splint oligonucleotide comprising a first portion and a second
portion, wherein a hybrid is formed wherein [[a]]the first portion of the splint oligonucleotide
hybridizes with the first portion of the first oligonucleotide and a second portion of the splint
oligonucleotide hybridizes with the first portion of the second oligonucleotide.

77. (Canceled)

78. (Currently Amended) A method of detecting an analyte, comprising:

(i) combining:

(a) an analyte;

(b) a first proximity member, comprising a first analyte-specific binding
entity and a first oligonucleotide comprising a first portion and a second portion comprising a 5'
terminus wherein the first analyte-specific binding entity is capable of forming a complex with
the analyte and is conjugated to the first oligonucleotide;

(c) a second proximity member, comprising a second analyte-specific
binding entity and a second oligonucleotide comprising a first portion wherein the second
analyte-specific binding entity is capable of forming a complex with the analyte and is
conjugated to the second oligonucleotide, wherein the first portion of the first oligonucleotide is

capable of hybridizing to the first portion of the second oligonucleotide and the ~~at least~~ first and/or second analyte-specific binding entity is a protein;

(ii) forming a complex comprising the analyte, the first proximity member, and the second proximity member, wherein the complex contains at least one hybrid comprising the first portion of the first oligonucleotide and the first portion of the second oligonucleotide, wherein the at least one hybrid comprises ~~[[a]]the~~ 3' terminus of the second oligonucleotide that is capable of being extended to form a complement of the second portion of the first oligonucleotide;

(iii) producing an amplicon comprises: (i) adding a third oligonucleotide and forming a hybrid between the second portion of the first oligonucleotide and the third oligonucleotide and (ii) ligating the 3' terminus of the second oligonucleotide to a 5' terminus of the third oligonucleotide;

(iv) amplifying the amplicon and producing an amplification product; and

(v) detecting the amplification product, wherein detection of the amplification product ~~allows~~ indicates detection of the analyte.

79. - 114. (Cancelled)